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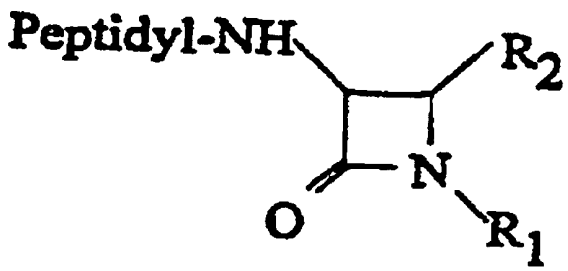
(54) **NOUVEAUX DERIVES 3-PEPTIDYL-AZETIDIN-2-ONE SUBSTITUEE EN 4 UTILES EN TANT QU'INHIBITEUR DES CYSTEINES PROTEINASES**

(54) **NOVEL 4-SUBSTITUTED-3-PEPTIDYL-AZETIDIN-2-ONE DERIVATIVES USEFUL AS CYSTEINE PROTEINASE INHIBITOR**



(I)

(57) Certains composés 3-peptidyl-azétidin-2-one substituée en 4 possèdent une excellente activité d'inhibition des cystéines protéinases, et on peut les utiliser dans le traitement de différentes maladies telles que la myopathie primitive progressive, la résorption osseuse, l'infarctus du myocarde et les métastases cancéreuses. Ces composés sont des 3-peptidyl-azétidin-2-ones substituées en 4 de la formule (I), ou des sels de celles-ci, acceptables sur le plan pharmacologique. Dans cette formule, R_1 représente hydrogène, alkyle C_1-C_6 substitué ou non; R_2 est choisi dans le groupe constitué par hydrogène, alkyle C_1-C_6 substitué ou non, -XR_6 où X représente O, S, SO ou SO_2 et R_6 représente alkyle C_1-C_6 substitué ou non. Le groupe peptidyle est un reste d'acide aminé 1-2 dans lequel le groupe libre NH_2 n'est pas substitué ou l'est par un groupe protecteur choisi dans le groupe constitué par aryloxy carbonyle, alcoxy

(57) Certain 4-substituted-3-peptidyl-azetidin-2-one compounds exhibit excellent cysteine proteinase inhibitory activity which can be used in the treatment of different diseases such as muscular dystrophy, bone resorption, myocardial infarction and cancer metastasis. These compounds are 4-substituted-3-peptidyl-azetidin-2-ones of formula (I) or pharmaceutically acceptable salts thereof, wherein R_1 is hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted; R_2 is selected from the group consisting of hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted; -XR_6 wherein X is O, S, SO, or SO_2; R_6 is C_1-C_6 alkyl which is unsubstituted or substituted. Peptidyl group is a 1-2 amino acid residue wherein the free NH_2 is unsubstituted or substituted with a protective group selected from the group consisting of aryloxy carbonyl, alkoxy carbonyl, substituted alkanoyl, arylalkanoyl, arylalkenoyl, heterocyclealkanoyl,

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carbonyle, alcanoyle substitué, arylalcanoyle, arylalcénoyle, hétérocycle-alcanoyle, hétérocyle-alcénoyle, alkylsulphonyle, arylsulphonyle, arylalcanylsulphonyle, arylalcènesulphonyle, hétérocycle-alcanylsulphonyle, hétérocycle-alcènesulphonyle et hétéroarylsulphonyle.

heterocyclealkenoyl alkylsulphonyl, arylsulphonyl, arylalkanylsulphonyl, arylalkensulphonyl, heterocyclealkanylsulphonyl, heterocyclealkensulphonyl, and heteroarylsulphonyl.

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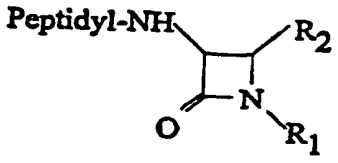

(I)

(57) Abstract

Certain 4-substituted-3-peptidyl-azetidin-2-one compounds exhibit excellent cysteine proteinase inhibitory activity which can be used in the treatment of different diseases such as muscular dystrophy, bone resorption, myocardial infarction and cancer metastasis. These compounds are 4-substituted-3-peptidyl-azetidin-2-ones of formula (I) or pharmaceutically acceptable salts thereof, wherein R_1 is hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted; R_2 is selected from the group consisting of hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted; -XR_6 wherein X is O, S, SO, or SO_2; R_6 is C_1-C_6 alkyl which is unsubstituted or substituted. Peptidyl group is a 1-2 amino acid residue wherein the free NH_2 is unsubstituted or substituted with a protective group selected from the group consisting of aryloxy carbonyl, alkoxy carbonyl, substituted alkanoyl, arylalkanoyl, arylalkenoyl, heterocyclealkanoyl, heterocyclealkenoyl alkylsulphonyl, arylsulphonyl, arylalkanylsulphonyl, arylalkensulphonyl, heterocyclealkanylsulphonyl, heterocyclealkensulphonyl, and heteroarylsulphonyl.

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Novel 4-substituted-3-peptidyl-azetidin-2-one derivatives useful as Cysteine proteinase inhibitor

Background of invention

Cysteine proteinases containing a highly reactive cysteine residue with a free thiol group at the active site have been known as playing important role in certain conditions distinguished by aberrant protein turnover such as: muscular dystrophy (Am. J. Pathol. 1986, 122, 193-198, Am. J. Pathol. 1987, 127, 461-466), bone resorption (Biochem. J. 1991, 279, 167-274), myocardial infarction (J. Am. Coll. Cardiol. 1983,2, 681-688), cancer metastasis (Cancer Metastasis Rev. 1990, 9, 333-352) and pulmonary emphysema (Am. Rev. Respir. Dis. 1975,111, 579-586). A variety of cysteine proteinases have been shown to be present in mammalian tissue. The most notable of these proteinases are the lysosomal cathepsins (cathepsin B, H, S, and L) and the cytoplasmic Ca^{2+} dependent enzymes, the calpains. These enzymes are, therefore, excellent targets for the development of specific inhibitors as possible therapeutic agents for the conditions such as those noted above.

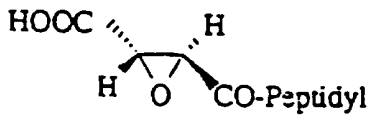
Cysteine proteinases are inhibited by several types of peptide derived inhibitors such as peptidyl aldehyde (Eur. J. Biochem. 1982, 129, 33-41), chloromethyl ketone (acta. Biol. Med. Ger. 1981, 40, 1503-1511), diazomethyl ketone (Biochemistry 1977,16, 5857-5861), monofluoromethyl ketone (Biochemical Pharmacology 1992 44, 1201-1207), acyloxy methyl ketone (J. Med. Chem. 1994, 37, 1833-1840), O-acyl hydroxamates (Biochem. Biophy. Research Communications 1988, 155, 1201-1206), methyl sulphonium salts (J. Biol. Chem. 1988, 263, 2768-2772) and epoxy succinyl derivatives (Agric. Biol. Chem. 1978, 42, 523-527) which do not significantly inhibit other classes of proteinases.

These inhibitors, in general, have peptidyl affinity groups and reactive groups towards the thiol of the cysteine residue of cysteine proteinase. Some of the

inhibitors are clinically useful. However, their effectiveness in vivo is not as much as expected on the basis of in vitro inhibitory activity, perhaps due to lower selectivity towards other proteinases and poor pharmacokinetics. Therefore, there exits a continuing need to develop new cysteine proteinase inhibitors with high selectivity and lower toxicity.

Peptidyl-CO-Y

Y = H, CH_2Cl, CHN_2, CH_2F, CH_2OCOAr, NHOCOR, CH_2S-$(CH_3)_2$



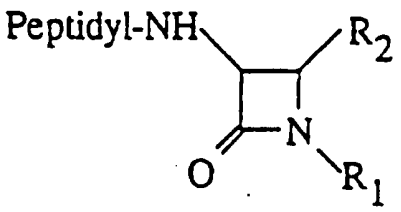
Epoxysuccinyl derivative

Summary of the invention

In a search for novel types of cysteine proteinase inhibitors with high selectivity for the cysteine proteinase class of enzymes, a novel class of compounds, having a peptidyl group at C-3 of reactive group 3-amino-4-substituted azetidin-2-one, represented by general formula I, have been found. These compounds exhibit an excellent cysteine proteinase inhibitory activity and selectivity among cysteine proteinases.

The present invention is based on the discovery that certain 4-substituted-3-peptidyl-azetidin-2-one derivatives exhibit excellent cysteine proteinase inhibitory activity which can be used for treatment of different diseases such as muscular dystrophy, bone resorption, myocardial infarction or cancer metastasis.

In accordance to the present invention, there is provided 4-substituted-3-peptidyl-azetidin-2-one derivatives of general formula I or pharmaceutically acceptable salts thereof,



wherein

R_1 is selected from the group consisting of hydrogen, C_1-C_6 alkyl unsubstituted or substituted with 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, carboxy and amino; $-OR_3$ wherein R_3 is a C_1-C_6 alkyl which may be substituted by 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, carboxy or amino; and $-SO_3$-M^+ wherein M is hydrogen, a metal ion which is selected from the group consisting of sodium, potassium, magnesium, and calcium, or $N^+(R_4)_4$ wherein R_4 is C_1-C_6 alkyl group;

R_2 is selected from the group consisting of hydrogen; C_1-C_6 alkyl, unsubstituted or substituted with 1-2 substituents selected from the group consisting of hydroxy, halogen,cyano, carboxy and amino; $-OCOR_5$ wherein R_5 is (i) a C_1-C_6 alkyl unsubstituted or substituted with 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, heterocycle, and amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, or (v) phenyl which is unsubstituted or substituted by 1-3 substituents selected from the group consisting of hydroxy, halogen, C_1-C_4 alkyl, C_1-C_2 alkoxy and or cyano; $-XR_6$ wherein X is O, S, SO,or SO_2 and R_6 is (i) C_1-C_6 alkyl unsubstituted or substituted by 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, heterocycle, and amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) phenyl which is unsubstituted or substituted with 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or substituted with at least one of carboxy or amino, C_1-C_2 alkoxy and cyano, or (vi) heterocycle which may be mono or bicyclic;

A peptidyl group is a 1-2 amino acid residue wherein the amine is unsubstituted or substituted with protective group R_7. R_7 is selected from the group consisting of hydrogen, $-COOR_8$ wherein R_8 is (i) C_1-C_6 alkyl which is unsubstituted or with phenyl, or (ii) phenyl; $-COR_9$ wherein R_9 is selected from the group consisting of (i) C_1-C_6 alkyl which is unsubstituted or substituted by 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, amino, 4-acetoxyphenyloxy, heterocycle, and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents selected from halogen, hydroxy, cyano, or amino, (ii) C_2-C_4 alkenyl is unsubstituted or substituted with heterocycle or phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents selected from halogen, hydroxy, cyano or amino, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) a phenyl group which is unsubstituted or substituted by 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or may be substituted with at least one of carboxy, or amino or both, C_1-C_2 alkoxy group or cyano, or (vi) a heterocycle which may be mono or bicyclic, $-SO_2R_{10}$ wherein R_{10} is selected from the group consisting of (i) C_1-C_6 alkyl, (ii) C_2-C_4 alkenyl which is unsubstituted or substituted with heterocycle or phenyl, (iii) phenyl which is unsubstituted or substituted with 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl group, C_1-C_2 alkoxy group and cyano, and (iv) naphthyl which is unsubstituted or substituted by 1-3 substituents selected from hydroxy, halogen,cyano, carboxy, C_1-C_4 alkyl, or C_1-C_2 alkoxy.

The pharmaceutically acceptable salts of formula I are selected from the group consisting of sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, fumaric acid and p-toluenesulfonic acid.

Examples of C_1-C_6 alkyl groups as substituents in R_1 R_2, R_3, R_4, R_5, R_6, R_8, R_9, or R_{10} are straight or branched

chain alkyl group having 1-6 carbon atoms such as methyl, ethyl, propyl, 1-methylethyl, butyl, 1-methylpropyl, 2-methylprop-1-yl, 2-methylprop-2-yl, pentyl, 3-methylbutyl, hexyl and the like.

Examples of halogen atoms as substituents in R_1 R_2, R_3, R_5, R_6, R_9,or R_{10} are fluorine, chlorine, bromine or iodine.

Examples of C_2-C_4 alkenyl group as defined in R_5, R_6, R_9, or R_{10} are alkenyl group having 2-4 carbon atoms such as ethenyl, 1-propenyl, 2-propenyl, 1-butenyl, 3-butenyl and the like.

Examples of C_2-C_4 alkynyl group as defined in R_5, R_6, R_9, or R_{10} are alkynyl group having 2-4 carbon atoms such as ethynyl, 1-propynyl, 2-propynyl, 1-butynyl, 3-butynyl and the like.

Examples of C_3-C_6 cycloalkyl groups as defined in R_5, R_6, or, R_9 are cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl.

Examples of heterocyclic group or substituents as defined in R_5, R_6, R_9, or R_{10} are C_2-C_{11} heterocyclic group which may have 1-3 heteroatoms selected from nitrogen, sulpher or oxygen. Preferred heterocyclic groups are thiophene, pyridine, 1,2,3-triazole, 1,2,4-triazole, quinoline, benzofuran, benzothiophene, morpholine, thiomorpholine, piperazine, piperidine and the like.

Examples of C_1-C_4 alkyl groups as substituents in R_5, R_6, R_9, or R_{10} are methyl, ethyl, propyl, 2-methyl propyl, butyl, 1,1-dimethyl ethyl and the like.

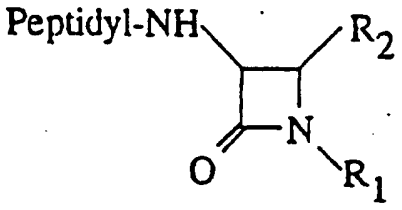
Examples of C_1-C_2 alkoxy group as substituents in R_5, R_6, R_9, or R_{10} are methoxy or ethoxy.

The term "amino acid residue" used herein refers to the remaining group after the removal of the hydroxy group from a carboxy group of an amino acid. The term "1-2 amino acid" used herein is one amino acid or one dipeptide consisting of two amino acids which are bonded to each other through a peptide bond.

Examples of amino acids are α-amino acids which are the constituents of normal protein, or their optical isomers, such as glycine, D- or L-alanine, D- or L-valine, D- or L-leucine, D- or L-isoleucine, D- or L-serine, D- or L-threonine, D- or L-aspartic Acid, D- or L-glutamic acid, D- or L-asparagine, D- or L-glutamine, D- or L-lysine, D- or L-arginine, D- or L-phenylalanine, D- or L-phenyl glycine, D- or L-tyrosine, D- or L-methionine, D- or L-hydroxy tyrosine, D- or L-proline and the like.

The azetidinone nucleus carries two asymmetric carbon atoms at position 3 and 4, and can therefore exist as 4-diastereoisomers. In general, the preferred isomer is that in which the hydrogen atoms at C3 and C4 are trans to each other this isomer has superior inhibitory activity against different cysteine proteinases such as papain, Cathepsin B, Cathepsin H and Cathepsin L. Such diasterioisomers and their racemic mixtures are also included within use of the azetidinone derivatives as cystein proteinase inhibitors.

A preferred embodiment of the invention provides 4-substituted-3-peptidyl-azetidin-2-one derivatives of general formula I



wherein:

R_1 is selected from the group consisting of hydrogen, methoxy, 2-carboxy ethoxy, 2-aminoethoxy, 2-carboxy ethyl, 2-aminoethyl and sulphonic acid.

R_2 is selected from the group consisting hydrogen, methyl, 2-amino ethyl, 2-carboxy ethyl, acetoxy, butyloxy, 3-methyl propyloxy, 1,1-dimethyl ethoxy, 2-carboxy ethyloxy, 2-aminoethyloxy, 2-fluoro ethoxy, 2-(1,2,3-triazol-4-yl)-ethoxy, cyclopentyloxy, cyclohexyloxy, cyclohexylthio, phenoxy, phenylthio, phenylsulphonyl, 4-(2-carboxy-2-amino ethyl)-phenoxy, 4-carboxy phenoxy, 3-

carboxy phenoxy, 2-pyridylthio, 4-pyridylthio and the like.

Peptidyl group is selected from the group consisting of phenylalanine, N-benzyloxy carbonyl phenylalanine, N-(3-phenyl propanoyl)-phenyl alanine, N-acetyl phenylalanine, N-{2-(4-acetoxyphenoxy)- ethanoyl}-phenyl alanine, N-(morpholin-4-yl-carbonyl)-phenyl alanine, N-{3-(morpholin-4-yl)-propanoyl}-phenyl alanine, N-{3-(pyridin-3-yl)-propanoyl}-phenyl alanine, N-(benzofuran-2-yl-carbonyl)-phenyl alanine, N-{3-(thiophen-2-yl)-prop-2-enoyl}-phenyl alanine, N-{4-(1,1-dimethyl ethyl phenyl)-sulphonyl}-phenyl alanine, N-(naphthalen-2-yl-sulphonyl)-phenyl alanine, N-(3-phenyl-prop-2-en-sulphonyl)-phenyl alanine, N-benzyloxy carbonyl leucine, N-benzyloxy carbonyl isoleucine, N-3-phenyl propanoyl leucine, N-3-phenyl propanoyl isoleucine, N-benzyloxy carbonyl proline, N-benzyloxy carbonyl phenyl alanine-glycine and the like.

More specifically, the most preferred embodiments of the present invention include the following compounds:

(3S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-1-methoxy-azetidin-2-one;

(3S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-azetidin-2-one;

(3R)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-1-methoxy-azetidin-2-one;

(3R)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-methyl-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl-glycyl)-amino-4-methyl-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-leucyl)-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-(N-acetyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(trans-3-phenylpropenoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(morpholin-4-yl-carbonyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-morpholin-4-yl-propionoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-pyrid-3-yl-propionoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-[N-{2-(4-acetoxyphenoxy)-ethnoyl}-L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(benzofuran-2-yl-carbonyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-[N-(3-(thiophen-2-yl)-trans-prop-2-enoyl}-L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-[N-{4-(1,1-dimethyl ethyl phenyl)-sulfonyl}-L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(naphthalen-2-yl-sulfonyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one;

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylsulfonyl-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenoxy-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-butyloxy-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(2-methyl propyloxy)-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(1,1-dimethylethoxy)-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-phenoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-(4-diphenylmethoxy carbonylphenoxy)-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-(4-carboxyphenoxy)-azetidin-2-one;

(3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-(3-carboxyphenoxy)-azetidin-2-one;

(3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-(4-(L-2-benzyloxy-carbonylamino-2-diphenylmethoxycarbonyl ethyl)-phenoxy)-azetidin-2-one;

(3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-(4-(L-2-amino-2-carboxy ethyl)-phenoxy)-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(4-diphenylmethoxycarbonyl phenoxy)-azetidin-2-one;

(3S,4S)-3-(L-phenylalanyl)-amino-4-(4-carboxyphenoxy)-azetidin-2-one;

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-alanyl)-amino-4-acetoxy-azetidin-2-one; and

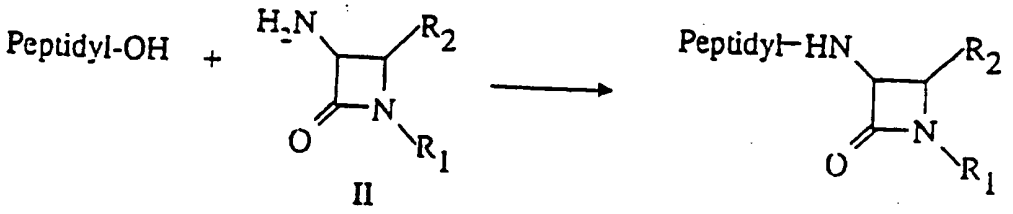
(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(pyrid-4-yl-thio)-azetidin-2-one;

Compounds of formula I may be utilized for treatment of different diseases, including muscular dystrophy, cancer metastasis and osteoporosis. The compounds of the invention are most useful to treat cancers which have a high tendency to metastasize, including breast, lung, liver, colon, brain, and prostate. Though not wishing to be restricted to any mechanism of action, the present invention is believed to work by inhibiting the cystein proteinase in medicaments formulated with pharmaceutically acceptable carriers and the compounds of the invention.

Description of Preferred Embodiments

The present invention relates to the certain 4-substituted-3-peptidyl-azetidin-2-one derivatives having excellent cystein proteinase inhibitory activity and selectivity among cystein proteinase enzymes. The compounds of this invention are characterized by having hydrogen, ester ($OCOR_6$), ether (OR_6), or thioether (SR_6) at

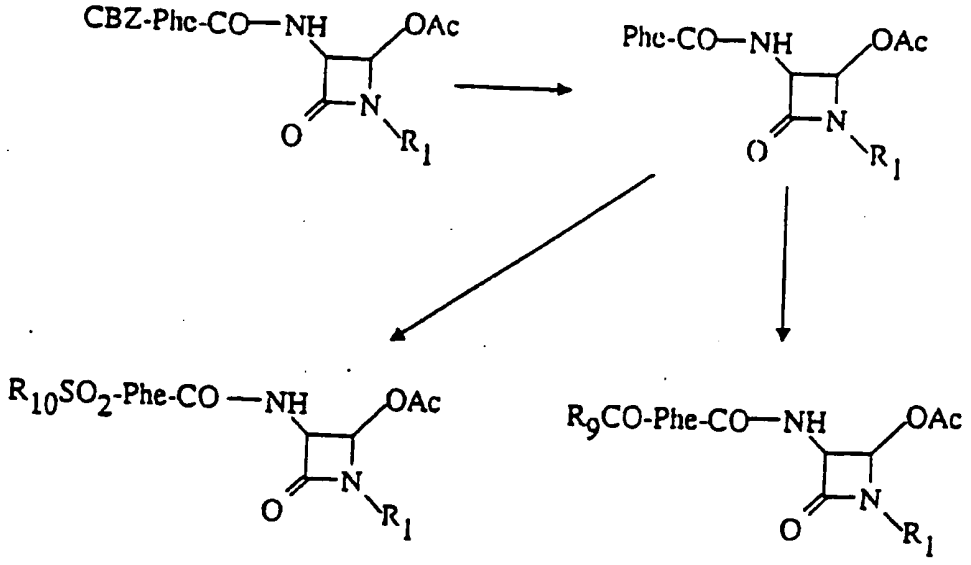
position 4 and substituted peptidyl group and peptidyl mimic group at position 3 of azetidin-2-one. Certain derivatives of general formula I were prepared by the common intermediates II by reacting with substituted peptidyl carboxylic acids either in presence of dicyclohexylcarbidiimide (DCC) or acid chloride in presence of base, or activated ester as shown in scheme I.



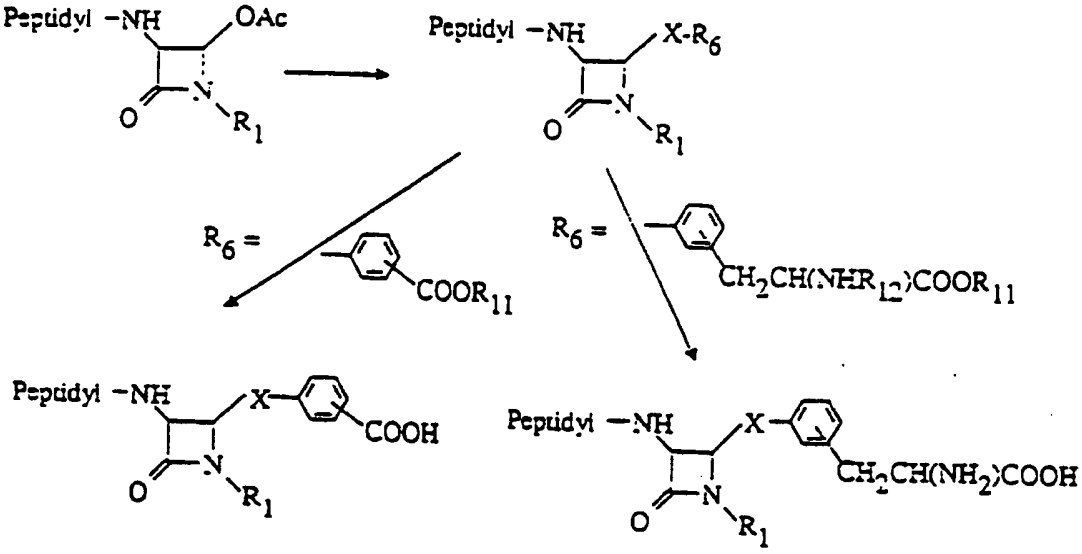
The preparation of compounds II were carried out by following the synthetic route as described in Eur. J. Med. Chem 1992, 27, 131-140, and Tetrahedron 1983, 39, 2577-2589.,wherein R_2 is hydrogen, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, or $OCOR_5$, and peptidyl group is a 1-2 amino acid residue with a protective group $COOR_8$. The definition of R_1, R_5 and R_8 are the same as defined above. The alkyl C_1-C_6 is unsubstituted or substituted with 1-2 substituents selected from the group consisting of halogen, hydroxy, cyano, amino, carboxy, heteroaryl and phenyl.

Certain 4-substituted-3-peptidyl-azetidin-2-one derivatives of general formula I wherein substitutions at the peptidyl group are other than $COOR_8$, such as COR_9 or SO_2R_{10} were prepared by following the synthetic route as shown in scheme II. The R_8, R_9 and R_{10} are same as defined above. The benzyloxycarbonyl protected peptidyl groups were deprotected and reprotected through amide bond by reaction with R_9-COOH, either in the presence of DCC, or reaction with acid chloride in the presence of base, or reaction with anhydride in the presence of base or activated ester, or through sulphonamide bond by reaction

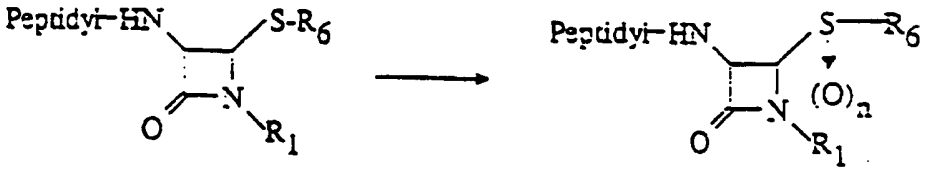
with $R_{10}SO_2Cl$ in the presence of base.



Certain 4-substituted-3-peptidyl-azetidin-2-one derivatives of general formula I wherein R_2 is XR_6, wherein X is O or S, and R_6 is same as defined above, were prepared by following the synthetic route as shown in scheme III starting from a compound of general formula I wherein R_2 is $OCOCH_3$. The compound of formula I is reacted with R_6XH in the presence of lewis acids such as zinc acetate, zinc iodide, zinc chloride, titanium tetrachloride, palladium acetate, boron trifluoride, aluminium trichloride and the like. In certain cases where a carboxy group as substituent in R_6 is protected with an R_{11} such as diphenyl methyl or 1,1-dimethyl ethyl, or where an amino group as substituent in R_6 is protected with an R_{12} such as benzyloxy carbonyl or 1,1-dimethyl ethoxy carbonyl, or where both protected groups as substituents in R_6 together were deprotected by hydrogenation or hydrolysis with acids.

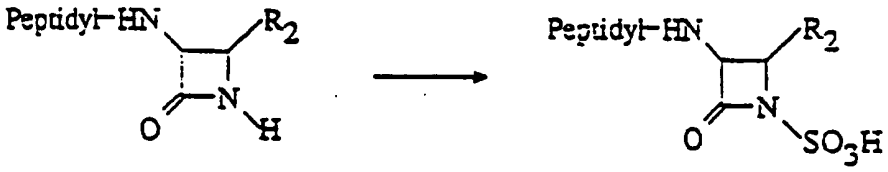


Certain 4-substituted-3-peptidyl-azetidin-2-one derivatives of general formula I wherein R_2 is SR_6 were converted to SOR_6 or SO_2R_6 by oxidation with an oxidizing agent selected from the group consisting of m-chloroperbenzoic acid, hydrogen peroxide. peracetic acid, potassium permanganate, manganese dioxide and the like. The synthetic route is outlined in scheme III.



n is 1 or 2

Alternatively, certain 4-substituted-3-peptidyl-azetidin-2-one derivatives of general formula I wherein R_1 is hydrogen were converted to N-sulphonic acid by sulphonation with pyridine-SO_3 or dimethylformamide-SO_3 complex. The synthetic route is outlined in scheme IV.



In the above processes, the reactants are reacted together with solvent at elevated or low temperatures for sufficient time to allow the reaction to proceed to completion. The reaction conditions will depend upon the nature and reactivity of the reactants. Wherever a base is used in a reaction, it is selected from the group consisting of triethylamine, pyridine, 4-dimethylaminopyridine, diisoipropylethylamine, 1,5-diazabicyclo[4,3,0]non-5-ene, 1,8-diazabicyclo [5,4,0]undec-7-ene, sodium carbonate, potassium carbonate and cesium carbonate.

Preferred solvents for the reaction are non reactive solvents. Depending on the reactants, a solvent will generally be selected from the group consisting of benzene, toluene, acetonitrile, tetrahydrofuran, ethanol, methanol, chloroform, ethyl acetate, methylene chloride, dimethyl formamide, dimethyl sulfoxide, hexamethyl phosphoric triamide, and the like. Solvent mixtures may also be utilized.

Reaction temperatures generally range from between -70^{0}C to 150^{0}C. The preferred molar ratio of reactants are 1:1 to 5.0. The reaction time ranges from 0.5 to 72 hours, depending on the reactants.

The deprotection of N-protective groups is carried out either by hydrogenation or by hydrolysis with appropriate acids such as hydrochloric acid, trifluoroacetic acid or acetic acid in solvent such as methanol, ethanol, propanol or ethyl acetate. The

hydrogenation reaction is usually carried out in the presence of a metal catalyst, such as Pd, Pt, or Rh, under normal pressure to high pressure.

The compounds of this invention, when used alone or in combination with other drugs as an agent for treating muscular dystrophy, osteoporosis or cancer metastasis in mammals including humans, may take pharmaceutical dosage forms including parenteral preparation such as injections, suppositories, aerosols and the like, and oral preparations such as tablets, coated tablets, powders, granules, capsules, liquids and the like. Injections are generally preferred. The above preparations are formulated in a manner known in the art.

For the formulation of solid preparations for oral administration, an excipient, and if desired, a binder, disintegrator, lubricant, coloring agent, corrigent, flavor, etc. is added to the compound of the invention, and then tablets, coated tablets, granules, powders, capsules or the like are prepared in a conventional manner.

For the formulation of injections, a pH adjusting agent, buffer, stabilizer, isotonic agent,local anesthetic or the like is added to the active ingredient of the invention. Injections for subcutaneous, intramuscular or intravenous administration can be prepared in the conventional manner.

For the formulation of suppositories, a base, and, if desired, a surfactant are added to the active ingredient of the invention, and the suppositories are prepared in a conventional manner.

The excipients useful for solid preparations for oral administration are those generally used in the art, such as lactose, sucrose, sodium chloride, starches, calcium carbonate, kaolin, crystalline cellulose, methyl cellulose, glycerin, sodium alginate, gum arabic and the like. Other ingredients which may be used in the formulations of the invention include binders such as polyvinyl alcohol, polyvinyl ether, polyvinyl pyrrolidone,

ethyl cellulose, gum arabic, shellac, sucrose, water, ethanol, propanol, carboxymethyl cellulose, potassium phosphate and the like; lubricants such as magnesium stearate, talc and the like; and additives such as usual known coloring agents, disintegrators and the like. Examples of bases useful for the formulation of suppositories are oleaginous bases such as cacao butter, polyethylene glycol, lanolin, fatty acid triglycerides, witepsol (trademark, Dynamite Nobel Co. Ltd.) and the like. Liquid preparations may be in the form of aqueous or oleaginous suspensions, solutions, syrups, elixirs and the like, which can be prepared by a conventional way using additives.

The amount of the compound of formula I of the invention to be incorporated into the pharmaceutical composition of the invention varies with the dosage form, solubility and chemical properties of the compound, administration route, administration scheme and the like. Preferably the amount is about 1 to 25 w/w% in the case of oral preparations, and about 0.1 to about 5 w/w% in the case of injections which are parenteral preparations.

The dosage of the compound I of the invention is suitably determined depending on the individual cases taking symptoms, age and sex of the subject and the like into consideration. Usually the dosage in the case of oral administration is about 50 to 1500 mg per day for an adult in 2 to 4 divided doses, and the dosage in the case of injection, for example, by intravenous administration is 2 ml (about 1 to 100 mg) which is administered once a day for adults wherein the injection may be diluted with physiological saline or glucose injection liquid if so desired, and slowly administered over at least 5 minutes. The dosage in case of suppositories is about 1 to 1000 mg which is administered once or twice a day at an interval of 6 to 12 hours wherein the suppositories are administered by insertion into the rectum.

Example 1

(3S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-1-methoxy-azetidin-2-one(1)

A solution of N-(benzyloxycarbonyl)-L-phenylalanine (150 mg, 0.5 mmol) in CH_2Cl_2 (10 ml) at - 5 °C was treated with triethylamine (0.077 ml, 0.55 mmol) and ethylchloroformate (0.05 ml, 0.5 mmol). The solution was stirred at 0 °C for 30 mins, and treated with (3S)-3-amino-1-methoxy-azetidin-2-one, trifluoroacetic acid salt (115 mg, 0.5 mmol) and pyridine (0.08 ml, 1.0 mmol). The resulting solution was stirred at room temperature overnight. The solvent was removed, and the residue was dissolved in Ethyl acetate (50 ml). The organic layer was washed with cold water (20 ml), brine and dried over sodium sulfate. After removal of solvent, the residue was triturated with ether/hexane (1/1) and gave a pale yellow syrup (130 mg).

Yield : 66%

^1H NMR ($CDCl_3$), δ (ppm) : 2.94-3.08 (2H, m), 3.67-3.70 (4H, m), 4.14 (1H, m), 4.38-4.32 (2H, m), 4.92 (1H, d, J = 12.4 Hz), 4.99 (1H, d, J = 12.4 Hz), 5.40 (1H, d, J = 7.9 Hz), 7.03-7.26 (11H, m).

Example 2

(3S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-azetidin-2-one(2)

In a similar manner to the method described in example 1, the title compound was obtained by reacting (3S)-3-amino-azetidin-2-one, trifluoroacetic acid salt with the ethoxy anhydride of N-(benzyloxycarbonyl)-L-phenylalanine.

Yield : 94%

^{1}H NMR ($CDCl_3$), δ (ppm) : 3.10 (2H, m), 4.45 (1H, m), 4.57 (1H, m), 5.00-5.15 (3H, m), 5.30 (1H, s), 5.65 (1H, bs), 7.05-7.48 (11H, m), 8.66 (1H, s).

Example 3

(3R)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-1-methoxy-azetidin-2-one(3)

In a similar manner to the method described in example 1, the title compound was obtained by reacting (3R)-3-amino-1-methoxy-azetidin-2-one, trifluoroacetic acid salt with the ethoxy anhydride of N-(benzyloxycarbonyl)-L-phenylalanine.

Yield : 93%

^{1}H NMR ($CDCl_3$), δ (ppm) : 2.99 (1H, s), 3.03 (1H, s), 3.65-3.17 (5H, m), 4.10 (1H, m), 4.64 (1H, m), 5.00 (2H, s), 5.37 (1H, bs), 6.78 (1H, d, J = 6.8 Hz), 7.23 (10 H, m).

Example 4

(3R)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-azetidin-2-one(4)

IN a similar manner to the method described in example 1, the title compound was obtained by reacting (3R)-3-amino-azetidin-2-one, trifluoroacetic acid salt with the ethoxy anhydride of N-(benzyloxycarbonyl)-L-phenylalanine.

Yield : 10 %

^{1}H NMR ($CDCl_3$), δ (ppm) : 3.11 (3H, m), 4.63 (1H, m), 5.07 (3H, m), 5.30 (1H, m), 7.05-7.40 (13H, m)

Example 5

Potassium (3S, 4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-methyl-azetidin-2-one-1-sulfonate(5)

A mixture of potassium (3S, 4S)-3-amino-4-methyl-azetidin-2-one-1-sulfonate (162 mg, 0.744 mmol), N-(benzyloxycarbonyl)-L-phenylalanine (223 mg, 0.744 mmol), DCC (153 mg, 0.744 mmol) and HOBt (100 mg, 0.744 mmol) in DMF (10 ml) was stirred at r.t overnight. DMF was removed in vacuum, and the residue was taken up in water (50 ml) and washed with methyl isobutyl ketone (3 x 50 ml) and hexane (50 ml). The aqueous portion was freeze-dried and purified by reversed-phase HPLC, giving an analytically pure white solid (49 mg).

Yield : 13%

m.p. : 300 °C(dec.)

Negative FAB-MS : 460 $(M-K)^-$, calcd for $C_{21}H_{22}O_7N_3SK$ 499

IR(KBr, cm^{-1}): 3285, 1760, 1700, 1670,1530, 1240, 1040

^1H NMR (D_2O), δ (ppm) : 1.45 (3H, d, J = 6.3 Hz), 3.03 (2H, m), 4.02 (1H, m), 4.34 (2H, m), 5.01 (1H, d, J = 12.5 Hz), 5.11 (1H, d, J = 12.5 Hz), 7.24-7.40 (10 H, m).

Example 6

Potassium (3S, 4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl-glycinyl)-amino-4-methyl-azetidin-2-one-1-sulfonate (6)

In a manner analogous to the method described in example 5, the title compound was obtained by using CBZ-Phe-Gly-OH as a starting material.

Yield : 11%

m.p. : 300 °C (dec.)

Negative FAB-MS : 517 $(M-K)^-$, calcd for $C_{23}H_{25}O_8N_4SK$ 556

IR (KBr, cm^{-1}) : 3430, 1770, 1670, 1560, 1250

^{1}H NMR (D_2O), δ (ppm) : 1.49 (3H, d, J = 6.3 Hz), 2.96 (1H, dd, J = 9.0 & 13.9 Hz), 3.17 (1H, dd, J = 6.2 & 13.9 Hz), 3.82 (1H, d, J = 17.1 Hz), 3.95 (1H, d, J = 17.1 Hz), 4.10 (1H, m), 4.40 (2H, m), 5.09 (1H, d, J = 12.5 Hz), 5.11 (1H, d, J = 12.5 Hz), 7.24-7.43 (10 H, m).

Example 7

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-4-acetoxy-azetidin-2-one (7)

(3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (5.56 g, 20 mmol) was hydrogenated with 5 g of 10% palladium on activated carbon in 100 ml of ethyl acetate at 50 psi hydrogen pressure at room temperature for 1.5 hrs. After removal of catalyst by filtration, deprotected (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate was obtained.

To a solution of N-benzyloxycarbonyl-L-phenylalanine (5.98 g, 20 mmol) and triethylamine (2.02 g, 20 mmol) in chloroform (100 ml), ethyl chloroformate (2.18 g, 20 mmol) was added at -15 °C. The reaction mixture was stirred at a bath temperature of -10 to 5 °C for 1.5 hrs. Then a precooled (ca. -15 °C) solution of (3S,4S)-3-amino-4-acetoxy-azetidin-2-one in ethyl acetate, which was obtained from hydrogenation of (3S,4S)-3-benzyloxycarbonylamino-4-acetoxy-azetidin-2-one (see above), was added at -15 °C and stirring was continued at a bath temperature of -15 to 5 °C for 1 hr. After removal of solvent, the residue was dissolved in ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent, the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:2) as eluent and the title compound was obtained as white solid.

Yield: 78 %

m.p. : 175-177 °C

FAB-MS: 426 (MH^+), calcd for $C_{22}H_{23}N_3O_6$ 425
IR (KBr, cm^{-1}) : 3315, 1797, 1740, 1680, 1660, 1533, 1258, 1227
^1H NMR(DMSO-d_6), δ (ppm): 2.10 (3H, s), 2.78 (1H, dd, J=14, 10), 3.02 (1H, dd, J=14, 4), 4.26 (1H, m), 4.64 (1H, d, J=8), 4.95 (2H, m), 5.76 (1H, s), 7.15-7.35 (10 H, m), 7.60 (1H, d, J=8), 8.83 (1H, d, J=8), 9.20 (1H, s).

Example 8

(3S,4S)-3-(N-benzyloxycarbonyl-L-leucyl) -amino-4-acetoxy-azetidin-2-one (8)

By a manner analogous to the method described in example 7, the title compound was obtained by reacting N-benzyloxycarbonyl-L-leucine with (3S,4S)-3-amino-4-acetoxy-azetidin-2-one.
Yield: 40 %
m.p. : 70-80 °C (dec.)
FAB-MS : 392 (MH^+), calcd for $C_{19}H_{25}N_3O_6$ 391
IR (KBr, cm^{-1}) : 3325, 1790, 1720, 1540, 1230, 1040
^1H NMR($CDCl_3$), δ (ppm) : 0.91 (6H, m), 1.48-1.68 (3H, m), 2.09 (3H, s), 4.27 (1H, m), 4.70 (1H, d, J = 7.4 Hz), 5.10 (2H, m), 5.66 (1H, bs), 5.80 (1H, s), 7.33 (6H, m), 7.59 (1H, bs).

Example 9

3S,4S)-3-(N-acetyl-L-phenylalanyl)-amino -4-acetoxy-azetidin-2-one (9)

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (850 mg, 2 mmol) obtained in example 7, was hydrogenated with 500 mg of 10% palladium on activated carbon in 60 ml of ethyl acetate at 50 psi hydrogen pressure at room temperature for 4 hrs in the presence of acetic anhydride (255 mg, 2.5

mmol). After filtration of the catalyst and removal of solvent, a white solid was collected and washed with ethyl acetate, diethyl ether and dried in air. 600 mg of title compound was obtained as white solid.

Yield: 90%

m.p. : 190-191 °C

FAB-MS: 334 (MH^+), calcd for $C_{16}H_{19}N_3O_5$ 333

IR (KBr, cm^{-1}) : 3380, 1800, 1751, 1647, 1529, 1370, 1219

^1H NMR(DMSO-d_6), δ (ppm): 1.77 (3H, s), 2.09 (3H, s), 2.75 (1H, dd, J=14, 10), 3.01 (1H, dd, J=14, 5), 4.49 (1H, m), 4.59 (1H, dd, J=8, 1), 5.74 (1H, d, J=1), 7.15-7.30 (5H, m), 8.15 (1H, d, J=8), 8.72 (1H, d, J=8), 9.16 (1H, s).

Example 10

(3S,4S)-3-(N-(3-phenylpropionoyl) -L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (10)

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (1.70 g, 4 mmol) obtained in example 7, was hydrogenated with 3.5 g of 10% palladium on activated carbon in 200 ml of ethyl acetate at 50 psi hydrogen pressure at room temperature for 2 hrs. After removal of catalyst by filtration, the deprotected (3S,4S)-3-(L-phenylalanyl)-amino -4-acetoxy-azetidin-2-one in ethyl acetate was obtained.

To a solution of 3-phenylpropionic acid (630 mg, 4 mmol) and triethylamine (425 mg, 4.2 mmol) in chloroform (80 ml), ethyl chloroformate (436 mg, 4 mmol) was added at -15 °C. The reaction mixture was stirred at a temperature of -10 to 5 °C for 2 hrs. Then a precooled (ca. -15 °C) solution of (3S,4S)-3-(L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one in ethyl acetate, which was obtained from hydrogenation of (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (see above), was added at -15 °C under

stirring at a bath temperature of -15 to 5 °C. The resulting solution was stirred for 1 hr and concentrated. The residue was dissolved in ethyl acetate, washed with a saturated solution of $NaHCO_3$, water, brine and dried over sodium sulfate. After removal of solvent, the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:2) as eluent and the title compound (1.1 g) was obtained as a white solid.

Yield: 65%

m.p. : 144.5-146.2 °C

FAB-MS: 424 (MH^+), calcd for $C_{23}H_{25}N_3O_5$ 423

IR (KBr, cm^{-1}) : 3380, 1803, 1749, 1644, 1535, 1218

^1H NMR(DMSO-d_6), δ (ppm): 2.09 (3H, s), 2.36 (2H, m), 2.68 (2H, m), 2.75 (1H, dd, J=14, 10), 3.01 (1H, dd, J=14, 5), 4.53 (1H, m), 4.60 (1H, dd, J=8, 1), 5.75 (1H, d, J=1), 7.05-7.30 (10H, m), 8.15 (1H, d, J=8), 8.72 (1H, d, J=8), 9.17 (1H, s).

Example 11

(3S,4S)-3-(N-(trans-3-phenylpropenoyl)-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (11)

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (200 mg, 0.47 mmol) obtained in example 7, was hydrogenated with 300 mg of 10% palladium on activated carbon in 50 ml of ethyl acetate at 50 psi hydrogen pressure at room temperature for 2 hrs. After removal of catalyst by filtration, the deprotected (3S,4S)-3-(L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one in ethyl acetate was cooled to -15 °C. Then triethylamine (50 mg, 0.5 mmol) and trans-β-styrenesulfonyl chloride (95 mg, 0.47 mmol) were added at -15 °C. Stirring was continued at a bath temperature of -10 to 5 °C for 2 hr. The reaction mixture was diluted with ethyl acetate, washed with water, brine and dried over sodium sulfate. After removal of solvent,

the residue was purified by silica gel column chromatography using hexane-ethyl acetate (1:1) as eluent and the title compound (200 mg) was obtained as a white solid.

Yield: 93%

m.p. : 103-105 °C

IR (KBr, cm-1) : 3315, 1785, 1748, 1672, 1523, 1321, 1227

^{1}H NMR(DMSO-d_6), δ (ppm): 2.03 (3H, s), 2.77 (1H, dd, J=14, 10), 2.92 (1H, dd, J=14, 5), 3.99 (1H, m), 4.57 (1H, d, J=8), 5.59 (1H, s), 6.55 (1H, d, J=16), 7.10-7.55 (11H, m), 7.94 (1H, d, J=8), 8.86 (1H, d, J=8), 9.19 (1H, s).

Example 12

(3S,4S)-3-(N-(morpholin-yl-carbonyl)-L-phenylalaninyl)-amino-4-acetoxy-azetidin-2-one (12)

By a method similar to the method described in example 7, the title compound was obtained by reacting N-(morpholin-yl-carbonyl)-L-phenylalanine with (3S,4S)-3-amino-4-acetoxy-azetidin-2-one.

Yield: 10 %

m.p. : 160.7-162.3 °C

FAB-MS: 405 (MH^+), calcd for $C_{19}H_{24}N_4O_6$ 404

IR (KBr , cm^{-1}) : 3380, 1787, 1748, 1668, 1623, 1535, 1224

^{1}H NMR(DMSO-d_6), δ (ppm): 2.09 (3H, s), 2.83 (1H, dd, J=14, 10), 3.01 (1H, dd, J=14, 4), 3.21 (4H, m), 3.45 (4H, m), 4.35 (1H, m), 4.64 (1H, d, J=1), 5.77 (1H, d, J=1), 6.65 (1H, d, J=8), 7.15-7.28 (5H, m), 8.67 (1H, d, J=8), 9.17 (1H, s).

Example 13

(3S,4S)-3-(N-(3-morpholin-4-yl-propionoyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (13)

By a method similar to the method described in example 10, the title compound was obtained by reacting 3-morpholin-4-yl-propionic acid with (3S,4S)-3-(L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.
Yield: 38%
m.p. : 85 °C (dec.)
FAB-MS : 433 (MH^+), calcd for $C_{21}H_{28}N_4O_6$ 432
IR (KBr , cm^{-1}) : 3285, 1780, 1750, 1650, 1540, 1450, 1370, 1220
^1H NMR($CDCl_3$), δ (ppm): 2.12 (3H, s), 2.40 (8H, m), 3.03 (1H, dd, J = 9.2 & 13.8 Hz), 3.22 (1H, dd, J = 5.1 & 13.8 Hz), 3.60 (4H, m), 4.61 (1H, d, J = 6.4 Hz), 4.75 (1H, dd, J = 7.8 & 14.0 Hz), 5.86 (1H, s), 7.0 (1H, s), 7.26 (5H, m), 7.49 (1H, d, J = 7.7 Hz), 8.83 (1H, d, J = 7.5 Hz).

Example 14

(3S,4S)-3-(N-(3-pyrid-3-yl-propionoyl)-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (14)

By a method similar to the method described in example 10, the title compound was obtained by reacting 3-(pyrid-3-yl)-propionic acid with (3S,4S)-3-(L-phenylalanyl) amino-4-acetoxy-azetidin-2-one.
Yield: 30%
m.p. : 150 °C (dec.)
FAB-MS : 425 (MH^+), calcd for $C_{22}H_{24}N_4O_5$ 424
IR (KBr , cm^{-1}) : 3310, 1790, 1740, 1660, 1540, 1370, 1230
^1H NMR(DMSO- d_6), δ (ppm) : 2.10 (3H, s), 2.40 (2H, t, J = 7.7 Hz), 2.72 (2H, t, J = 7.7 Hz), 2.82 (1H, dd, J = 9.4 & 14.0 Hz), 3.00 (1H, dd, J = 5.2 & 13.9 Hz),

4.54 (1H, m), 4.60 (1H, d, J = 8.4 Hz), 5.74 (1H, s), 7.22 (6H, m), 7.50 (1H, d, J = 7.0 Hz), 8.18 (1H, d, J = 8.8 Hz), 8.37 (2H, m), 8.74 (1H, d, J = 7.8 Hz), 9.18 (1H, s).

Example 15

(3S,4S)-3-[N-(2-(4-acetoxyphenoxy)-ethanoyl) -L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one (15)

BY a method similar to the method described in example 10, the title compound was obtained by reacting 4-acetoxyphenoxy acetic acid with (3S,4S)-3-(L-phenylalanyl) amino-4-acetoxy-azetidin-2-one.

Yield: 34 %

m.p. : 190 °C

FAB-MS: 506 (MNa^+), calcd for $C_{24}H_{25}N_3O_8$ 483

IR (KBr , cm^{-1}) : 3295, 1800, 1660, 1600, 1530, 1225

^1H NMR(DMSO-d_6), δ (ppm) : 2.10 (3H, s), 2.52 (3H, s), 2.98 (1H, dd, J = 9.2 & 13.8 Hz), 3.09 (1H, dd, J = 5.2 & 13.8 Hz), 4.56 (2H, s), 4.58 (1H, m), 4.63 (1H, d, J = 8.1 Hz), 5.76 (1H, s), 6.89 (2H, d, J = 8.8 Hz), 7.23 (5H, s), 7.87 (2H, d, J = 8.8 Hz), 8.33 (1H, d, J = 8.5 Hz), 8.83 (1H, d, J = 8.5 Hz), 9.20 (1H, s).

Example 16

(3S,4S)-3(N-(benzofuran-2-yl-carbonyl)-L-phenylalanyl) -amino-4-acetoxy-azetidin-2-one (16)

By a method similar to the method described in example 10, the title compound was obtained by reacting 2-benzofurancarboxylic acid with (3S,4S)-3-(L-phenylalanyl) amino-4-acetoxy-azetidin-2-one.

Yield: 50 %

m.p. : 115 °C (dec.)

FAB-MS: 436 (MH^+), calcd for $C_{23}H_{21}N_3O_6$ 435

IR (KBr , cm^{-1}) : 3295, 1790, 1750, 1650, 1520, 1370, 1220

^1H NMR($CDCl_3$), δ (ppm) : 2.03 (3H, s), 3.23 (2H, m), 4.75 (1H, d, J = 8.0 Hz), 5.07 (1H, dd, J = 5.8 & 13.8 Hz), 5.77 (1H, s), 7.23 (5H, m), 7.47 (3H, m), 7.60 (3H, m), 8.08 (1H, d, J = 6.8 Hz).

Example 17

(3S,4S)-3-[N-(3-(thiophen-2-yl)-trans-prop-2-enoyl) -L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one (17)

By a method similar to the method described in example 10, the title compound was obtained by reacting 2-thiopheneacrylic acid with (3S,4S)-3-(L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.

Yield: 54%

m.p. : 220-221 °C

FAB-MS: 428 (MH^+), calcd for $C_{21}H_{21}N_3O_5S$ 427

IR (KBr , cm^{-1}) : 3285, 1775, 1750, 1640, 1620, 1540, 1210

^1H NMR(DMSO-d_6), δ (ppm) : 2.07 (3H, s), 2.80 (1H, dd, J = 9.2 & 13.8 Hz), 3.05 (1H,dd, J = 5.1 & 13.8 Hz), 4.60 (1H, d, J = 8.4 Hz), 4.62 (1H, m), 5.75 (1H, s), 6.44 (1H, d, J = 14.7 Hz), 7.07 (1H, d, J = 4.2 Hz), 7.23 (5H, m), 7.34 (1H, d, J = 4.2 Hz), 7.52 (1H, d, J = 14.7 Hz), 7.60 (1H, d, J = 4.7 Hz), 8.42 (1H, d, J = 8.8 Hz), 8.82 (1H, d, J = 7.8 Hz), 9.16 (1H, s).

Example 18

3S,4S)-3-[N-(4-(1,1-dimethyl ethyl) phenyl sulfonyl) -L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one (18)

By a method similar to the method described in example 11, the title compound was obtained by reacting 4-(1,1-dimethyl ethyl)-phenylsulfonyl chloride with

(3S,4S)-3-(L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.

Yield: 74 %

m.p. : 125 °C (dec.)

FAB-MS: 510 (MNa^+), calcd for $C_{24}H_{29}N_3O_6S$ 487

IR (KBr , cm^{-1}) : 3295, 1780, 1750, 1660, 1520, 1330, 1225

^1H NMR(Acetone-d_6), δ (ppm) : 1.34 (9H, s), 2.08 (3H, s), 2.84 (1H, dd, J = 9.2 & 13.8 Hz), 3.03 (1H, dd, J = 5.7 & 13.8 Hz), 4.10 (1H, m), 4.67 (1H, dd, J = 1.3 & 7.8 Hz), 5.81 (1H, d, J = 1.1 Hz), 6.67 (1H, d, J = 8.9 Hz), 7.13 (5H, m), 7.48 (2H, d, J = 8.6 Hz), 7.60 (2H, d, J = 8.6 Hz), 8.06 (1H, d, J = 7.7 Hz), 8.17 (1H, s).

Example 19

(3S,4S)-3-(N-(naphthalen-2-yl-sulfonyl)-L-phenylalanyl) -amino-4-acetoxy-azetidin-2-one (19)

By a method similar to the method described in example 11, the title compound was obtained by reacting 2-naphthalenesulfonyl chloride with (3S,4S)-3-(L-phenylalanyl) amino-4-acetoxy-azetidin-2-one.

Yield: 42 %

m.p. : 174-176 °C

FAB-MS: 482 (MH^+), calcd for $C_{24}H_{23}N_3O_6S$ 481

IR (KBr , cm^{-1}) : 3330, 1780, 1750, 1670, 1320, 1225

^1H NMR($CDCl_3$), δ_(ppm) : 2.09 (3H, s), 2.83 (1H, dd, J = 9.2 & 14.1 Hz), 3.06 (1H, dd, J = 4.7 & 14.1 Hz), 4.04 (1H, m), 4.83 (1H, d, J = 7.8 Hz), 5.90 (1H, s), 5.95 (1H, s), 6.78 (5H, m), 7.26 (1H, s), 7.48-7.98 (7H, m), 8.20 (1H, s).

Example 20

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-4-phenylthio-azetidin-2-one (20)

A mixture of (3S,4S)-3- (N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (500 mg, 1.18 mmol) obtained in example 7, thiophenol (117 mg, 1.07 mmol), and zinc acetate dihydrate (207 mg, 0.95 mmol) in a mixture of benzene (20 ml) and toluene (20 ml) was refluxed for 4 hrs using Dean-Stark water separator. After cooling, the reaction mixture was partitioned between ethyl acetate, containing a small volume of acetone, and water. The organic layer was washed with water, brine and dried over sodium sulfate. After removal of the solvent to dryness, a white solid was washed with dichloromethane and 410 mg of the title compound was obtained as a white solid.

Yield: 73 %

m.p. : 174-175.5 °C

FAB-MS: 476 (MH^+), calcd for $C_{26}H_{25}N_3O_4S$ 475

IR (KBr , cm^{-1}) : 3300, 1772, 1683, 1522, 1240

^1H NMR(DMSO-d_6), δ (ppm): 2.77 (1H, dd, J=14, 10), 3.02 (1H, dd, J=14, 5), 4.26 (1H, m), 4.58 (1H, dd, J=8, 2), 4.95 (3H, m), 7.10-7.50 (15H, m), 7.58 (1H, d, J=8), 8.90 (1H, d, J=8), 9.03 (1H, s).

Example 21

(3R,4S)-3- (N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylsulfonyl-azetidin-2-one (21)

A mixture of (3R,4S)-3- (N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one (540 mg, 1.136 mmol) obtained in example 20, and 3-chloroperoxybenzoic acid (588 mg, 3.42 mmol) in dichloromethane (400 ml) was stirred at room temperature for 9 hrs. After removal of dichloromethane, the

reaction mixture was partitioned between ethyl acetate and water, the organic layer was washed with water, brine, and dried over sodium sulfate. After removal of the solvent to dryness, a white solid was washed with dichloromethane and 450 mg of the title compound was obtained as a white solid.

Yield: 78 %

m.p. : 200 °C (dec.)

FAB-MS: 508 (MH^+), calcd for $C_{26}H_{25}N_3O_6S$ 507

IR (KBr , cm^{-1}) : 3310, 1800, 1680, 1525, 1300, 1240

^1H NMR(DMSO-d_6), δ (ppm) : 2.71 (1H, dd, J = 9.1 & 13.8 Hz), 2.96 (1H, dd, J = 5.0 & 13.8 Hz), 4.21 (1H, m), 4.93 (4H, m), 7.26 (10H, m), 7.60 (1H, d, J = 7.8 Hz), 7.55-7.94 (5H, m), 8.92 (1H, d, J = 7.8 Hz), 9.32 (1H, s).

Example 22

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-4-phenoxy-azetidin-2-one (22)

By a method similar to the method described in example 20, the title compound was obtained as a white solid by reacting phenol with (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.

Yield: 22%

FAB-MS: 460 (MH^+), calcd for $C_{26}H_{25}N_3O_5$ 459

IR (KBr , cm^{-1}) : 3325, 3190, 1776, 1711, 1664, 1545, 1241

^1H NMR(DMSO-d_6), δ (ppm) : 2.81 (1H, dd, J = 9.1 & 13.9 Hz), 3.05 (1H, dd, J = 5.1 & 13.9 Hz), 4.28 (1H, m), 4.70 (1H, d, J = 9.0 Hz), 4.98 (2H, s), 5.53 (1H, s), 7.15-7.35 (10H, m), 7.67 (1H, d, J = 8.4 Hz), 8.97 (1H, d, J = 8.9 Hz), 9.34 (1H, s).

Example 23

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-4-butyloxy-azetidin-2-one (23)

By a method similar to the method described in example 20, the title compound was obtained by reacting 1-butanol with (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.
Yield: 14 %
m.p. : 162-164 °C
FAB-MS: 440 (MH^+), calcd for $C_{24}H_{29}N_3O_5$ 439
IR (KBr , cm^{-1}) : 3300, 1790, 1690, 1660, 1540
^1H NMR($CDCl_3$), δ (ppm) : 0.89 (3H, t, J = 7.4 Hz), 1.28 (2H, m), 1.49 (2H, m), 3.10 (2H, d, J = 6.4 Hz), 3.43 (2H, m), 4.46 (1H, dd, J = 7.0 & 14.6 Hz), 5.06 (3H, m), 5.35 (2H, m), 6.55 (1H, bs), 6.72 (1H, bs), 7.15-7.40 (10H, m).

Example 24

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(2-methyl propyloxy)-azetidin-2-one (24)

By a method similar to the method described in example 20, the title compound was obtained by reacting 2-methyl-1-propanol with (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.
Yield: 7 %
^1H NMR(Acetone-d_6), δ (ppm) : 0.88 (6H, d = 6.6 Hz), 1.85 (1H, m), 2.94 (1H, dd, J = 9.6 & 13.8 Hz), 3.26 (1H, dd, J = 4.6 & 13.8 Hz), 3.29 (2H, d, J = 6.7 Hz), 4.57 (1H, m), 5.00 (2H, s), 5.15 (1H, d, J = 3.9 Hz), 5.30 (1H, m), 6.48 (1H, bd, J = 8.4 Hz), 7.17-7.37 (10H, m), 7.76 (1H, d, J = 9.1 Hz), 8.08 (1H, s).

Example 25

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl) -amino-4-(1,1-dimethyl ethoxy)-azetidin-2-one (25)

By a method similar to the method described in example 20, the title compound was obtained by reacting 1,1-dimethyl ethanol with (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.

Yield:14%

^{1}H NMR($CDCl_3$), δ (ppm): 1.17 (9H, s), 3.10 (2H, d, J = 6.8 Hz), 4.45 (1H, dd, J = 7.0 & 14.6 Hz), 5.08 (2H, s), 5.31 (3H, m), 6.39 (2H, s), 7.20-7.40 (10H, m).

Example 26

(3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl) -amino-4-phenoxy-azetidin-2-one (26)

A mixture of (3S,4S)-3-((N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (212 mg, 0.5 mmol) obtained in example 10, phenol (41 mg, 0.45 mmol), and zinc acetate dihydrate (110 mg, 0.5 mmol) in a mixture of benzene (8 ml) and toluene (8 ml) was refluxed for 5.5 hrs using Dean-Stark water separator. The reaction mixture was purified by silica gel column chromatography using hexane-ethyl acetate (2:1) as eluent and the title compound (50 mg) was obtained as a white solid.

Yield: 22 %

m.p. : 199-201 °C (dec.)

FAB-MS: 458 (MH^+), calcd for $C_{27}H_{27}N_3O_4$ 457

IR (KBr , cm^{-1}) : 3290, 1782, 1641, 1538, 1491, 1225

^{1}H NMR(DMSO-d_6), δ (ppm): 2.37 (2H, m), 2.55-3.10 (4H, m), 4.54 (1H, m), 4.64 (1H, d, J=8), 5.51 (1H, s), 6.80-7.40 (15H, m), 8.23 (1H, d, J=8), 8.85 (1H, d, J=8), 9.32 (1H, s).

Example 27

(3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-(4-(diphenylmethoxycarbonyl)-phenoxy)-azetidin-2-one (27A) and (3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-(4-carboxyphenoxy)-azetidin-2-one (27B)

By a method similar to the method described in example 26, the protected title compound (27A) was obtained as a white solid by reacting 4-(diphenylmethoxycarbonyl)-phenol with (3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one prepared from example 10.

300 mg of the protected compound was hydrogenated with 600 mg of 5% palladium on activated carbon in 30 ml ethyl acetate at 50 psi hydrogen pressure at room temperature for 3 hrs. The catalyst was filtered and washed with ethyl acetate, and the combined filtrates were evaporated in vacuo. The residue was triturated with ether and the supernatant was decanted. The remaining solid was dried under vacuum to give white solid (120 mg).

The title compound (27B) was converted to sodium salt with $NaHCO_3$ (1 equivalent) in CH_3CN/H_2O for 0.5 h followed by freeze-drying.

Yield: 15 %

m.p. : 217 °C (dec.)

IR (KBr , cm^{-1}) : 3400, 3290, 1700, 1650, 1600, 1540, 1380, 1230

^1H NMR(DMSO-d_6), δ (ppm) : 2.39 (2H, t, J = 7.7 Hz), 2.73 (2H, t, J = 7.7 Hz), 2.80 (1H, dd, J = 9.2 & 13.8 Hz), 3.05 (1H, dd, J = 5.1 & 13.8 Hz), 4.51 (1H, m), 4.79 (1H, d, J = 8.4 Hz), 5.6 (1H, s), 6.76 (2H, t, J = 8.6 Hz), 7.2 (10H, m), 7.86 (2H, d, J = 8.6Hz), 8.28 (1H, d, J = 7.9 Hz), 9.4 (2H, s), 9.5 (1H, s).

Example 28

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl} -amino-4-(3-carboxyphenoxy)-azetidin-2-one (28)

By a method similar to the method described in example 27, the title compound (28) was obtained as a white solid by reacting 3-(diphenylmethoxycarbonyl)-phenol with (3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one following deprotection of the diphenylmethyl group.

Yield: 8.6%

m.p. : 190 °C (dec.)

Negative FAB-MS: 500 $(M-H)^-$, calcd for $C_{28}H_{27}N_3O_6$ 501

IR (KBr , cm^{-1}) : 3410, 3285, 1770, 1650, 1560, 1380, 1230

^1H NMR(DMSO-d_6), δ (ppm): 2.37 (2H, t, J = 7.7 Hz), 2.73 (2H, t, J = 7.7 Hz), 2.84 (1H, dd, J = 9.2 & 13.8 Hz), 3.10 (1H, dd, J = 5.1 & 13.8 Hz), 4.57 (1H, m), 4.80 (1H, d, J = 8.4 Hz), 5.6 (1H, d, J = 5.8 Hz), 6.83 (1H, d, J = 7.9 Hz), 7.2 (12H, m), 7.47 (1H, d, J = 11.3), 9.4 (2H, s).

Example 29

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-{4-(L-2-N-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenoxy}-azetidin-2-one (29A) and (3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-{4-(L-2-amino-2-carboxy-ethyl)-phenoxy}-azetidin-2-one (29B)

By a method similar to the method described in example 26, the protected title compound (29A) was obtained as a white solid by 4-(L-2-N-benzyloxycarbonylamino-2-diphenylmethoxycarbonyl-ethyl)-phenol with (3S,4S)-3-(N-(3-phenylpropionoyl)-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.

Yield: 28 %

^{1}H NMR(DMSO-d_6), δ (ppm): 2.36 (2H, m), 2.55-3.10 (6H, m), 4.35 (1H, m), 4.53 (1H, m), 4.60 (1H, d, J=8), 4.95 (2H, m), 5.45 (1H, s), 6.70-6.85 (3H, m), 7.00-7.40 (27H, m), 7.90 (1H, d, J=8), 8.20 (1H, d, J=8), 8.82 (1H, d, J=8), 9.30 (1H, s).

The protected compound, obtained above, was deprotected as described in example 27B and the title compound (29B) was obtained as a white solid.

Yield: 38 %

m.p. : 173-175 °C

FAB-MS: 545 (MH^+), calcd for $C_{30}H_{32}N_4O_6$ 544

IR (KBr , cm^{-1}) : 3405, 1771, 1649, 1507, 1226

^{1}H NMR(DMSO-d_6), δ (ppm): 2.38 (2H, m), 2.55-3.10 (6H, m), 3.85 (3H, br), 4.54 (1H, m), 4.64 (1H, d, J=8), 5.50 (1H, s), 6.80 (2H, d, J=8), 7.05-7.30 (12H, m), 8.38 (1H, d, J=8), 8.91 (1H, d, J=8), 9.35 (1H, s).

Example 30

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(4-(diphenylmethoxycarbonyl)-phenoxy)-azetidin-2-one (30A) and (3S,4S)-3-(L-phenylalanyl)-amino-4-(4-carboxyphenoxy)-azetidin-2-one (30B)

By a method similar to the method described in example 20, the protected title compound (30A) was obtained as a white solid by reacting 4-(diphenylmethoxycarbonyl) phenol with (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one prepared from example 7.

Yield: 26%

^{1}H NMR($CDCl_3$), δ (ppm): 3.10 (2H, m), 4.50 (2H, d, J = 7.4 Hz), 5.03 (2H, m), 5.51 (1H, bs), 5.78 (1H, s), 6.84 (2H, d, J = 8.8 Hz), 7.03-7.42 (23H, m), 8.08 (2H, d, J = 8.8 Hz).

The protected compound (30A), obtained above, was deprotected as described in example 27B and the title compound (30B) was obtained as a white solid.
Yield: 62%
m.p. : 180 °C (dec.)
Negative FAB-MS: 468 $(M-H)^-$, calcd for $C_{19}H_{19}N_3O_5$ 469
IR (KBr , cm^{-1}) : 3450, 1770, 1600, 1560, 1380, 1230
^1H NMR($CDCl_3$), δ (ppm): 2.69 (1H, dd, J = 8.9 & 13.3 Hz), 2.96 (1H, dd, J = 5.1 & 13.3 Hz), 3.48 (1H, t, J = 6.6 Hz), 4.66 (1H, s), 5.61 (1H, s), 6.83 (2H, d, J = 8.6 Hz), 7.23 (5H, s), 7.89 (2H, d, J = 8.6 Hz), 8.8 (1H, s), 9.3 (1H, s).

Example 31

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one-1-sulfonic acid (31)

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one (100 mg, 0.21 mmol) obtained in example 20 in DMF (3 ml) was cooled to 0 °C and SO_3-DMF (49 mg, 0.32 mmol) added. The reaction mixture was stirred at room temperature for 2 hrs. After removal of DMF under vacuum, a solution of KH_2PO_4 (44mg, 0.32 mmol) in 3 ml of water was added. After lyophilization, the solid was dissolved in water-acetonitril (1:1) and purified by reversed-phase thin-plate chromatography using water-acetonitril (2:8) as eluent. The title compound (90 mg) was obtained as a white solid after lyophilization.
Yield: 77 %
m.p. : 103-105 °C (dec.)
Negative FAB-MS: 554 $(M-H)^-$, calcd for $C_{26}H_{25}N_3O_7S_2$ 555
IR (KBr , cm^{-1}) : 3310, 1772, 1702, 1522, 1454, 1245
^1H NMR(DMSO-d_6), δ (ppm): 2.74 (1H, dd, J=14, 10), 3.01 (1H, dd, J=14, 4), 4.22 (1H, m), 4.51 (1H, dd, J=8, 2), 4.96 (3H, m), 7.10-7.40 (13H, m), 7.63 (2H, m), 7.52 (1H, d, J=8), 9.04 (1H, d, J=8).

Example 32

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one-1-sulfonic acid (32)

A solution of (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one (300 mg, 0.706 mmol) and sulfur trioxide pyridine complex (337 mg, 2.12 mmol) in anhydrous pyridine (5 ml) was refluxed for 40 mins. The mixture was cooled down and poured into KH_2PO_4 solution (0.5N, 50 ml). The aqueous solution was extracted with CH_2Cl_2 (2 x 25 ml) and the resulting organic phase was back-extracted with KH_2PO_4 solution (0.5N, 50 ml). The combined aqueous solution was treated with tetrabutylammonium hydrogen sulphate (240 mg, 0.706 mmol) and extracted with CH_2Cl_2 (3 x 30 ml). The combined organic extracts were dried over Na_2SO_4 and concentrated to pale yellow syrup. The crude product was subjected to flash column chromatography (silica gel, MeOH/Ethyl acetate : 1/9) to give a white solid (56 mg).

Yield : 16%

m.p. : 181 °C (dec.)

Negative FAB-MS: 504 $(M-H)^-$, calcd for $C_{22}H_{23}N_3O_9S$ 505

IR (KBr , cm^{-1}) : 3370, 1780, 1760, 1700, 1520, 1245

^1H NMR(CD_3CN/D_2O), δ (ppm): 2.06 (3H, s), 2.86 (1H, dd, J = 9.4 & 13.8 Hz), 2.89 (1H, dd, J = 5.2 & 13.8 Hz), 4.51 (1H, m), 4.55 (1H, s), 4,94 (1H, d, J = 16.0 Hz), 5.06 (1H, d, J = 16.0 Hz), 6.00 (1H, d, J = 10.1 Hz), 6.32 (1H, s), 7.23 (10H, m), 7.66 (J = 8.0 Hz).

Example 33

(3S,4S)-3-(N-benzyloxycarbonyl-L-alanyl)-amino-4-acetoxy-azetidin-2-one (33)

By a method analogous to the method described in example 7, the title compound was obtained by reacting N-benzyloxycarbonyl-L-alanine with (3S,4S)-3-amino-4-acetoxy-azetidin-2-one.

Yield: 53%

m.p. : 161-162 °C

FAB-MS: 350 (MH^+), calcd for $C_{16}H_{19}N_3O_6$ 349

IR (KBr , cm^{-1}) : 3360, 1770, 1690, 1665, 1520, 1230

^1H NMR($CDCl_3$), δ (ppm) : 1.36 (3H, d, J = 7.0 Hz), 2.09 (3H, s), 4.32 (1H, m), 4.67 (1H, d, J = 7.3 Hz), 5.05 (1H, d, J = 12.3 Hz), 5.13 (1H, d, J = 12.3 Hz), 5.78 (1H, d, J = 7.9 Hz), 5.83 (1H, s), 7.33 (5H, s), 7.53 (1H, bs).

Example 34

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(pyrid-4-yl) thio-azetidin-2-one (34)

By a method analogous to the method described in example 20, the title compound was obtained by reacting 4-mercaptopyridine with (3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one.

Yield: 8%

^1H NMR(DMSO-d_6), δ (ppm) : 2.80 (1H, m), 3.05 (1H, m), 4.30 (1H, m), 4.78 (1H, m), 4.96 (3H, m), 7.10-7.40 (12H, m), 8.90 (1H, d, J=8), 9.03 (1H, d, J=8), 9.22 (1H, s).

Testing of inhibitors for inhibition of Cathepsin B,L and papain.

In Vitro assay procedure for Cathepsin B

The compounds of formula I compounds were tested for inhibition of Cathepsin B. The procedure used was "A. J. Barret et al, Biochem.J.(1982), 201,189-198," with the following modifications To a 170 µl of enzyme-buffer mixture (enzyme:r rat CathB, diluted to give appr. 10 F units/min, buffer: 56mM Na acetate, 1.124mM EDTA, 10mM DTT, pH5.1) a 10 µl of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature a 20 µl of 5mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the Fluoroscan reader (excitation at 380nm, emission at 460nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained , and IC50 is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition). Of the compounds tested so far, the compounds of claim 1 wherein R_2 is hydrogen are the least active.

Test Example 2

Assay procedure for Cathepsin L

To a 170 µl of enzyme-buffer mixture (enzyme: r rat CathL, diluted to give appr 15 F units/min, buffer: 58.8mM Na citrate, 1.18mM EDTA, 235mM sodium chloride, 5mM DTT, pH5.0) a 10 µl of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature a 20 µl of 1mM substrate (N-CBZ-Phe-Arg-AMC, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 10 min at the Fluoroscan reader (excitation at 380nm, emission at 460nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC50 is determined using a

linear regression calculations (concentration of inhibitor which will give 50% inhibition).

Test Example 3

Assay procedure for papain

To a 170 µl of enzyme-buffer mixture (enzyme:papain, diluted to give 30mOD/min, buffer: 0.2M potassium phosphate, 1.0 mM EDTA, 5mML Cysteine, pH6.5) a 10 µl of inhibitor (dissolved in DMSO) was added. After 10 min of incubation at room temperature, 20 µl of 10mM substrate (N-CBZ-Pro-Phe-Arg-pNA, dissolved in DMSO) was added to initiate reaction. Reading is followed up for 3 min at the Thermomax plate reader(absorbance at 405 nm).

A plot of percentage of inhibition vs inhibitor concentration is obtained, and IC50 is determined using a linear regression calculations (concentration of inhibitor which will give 50% inhibition).

Table Of IC50 Values (µM)

Exampl	Cathepsin B	Cathepsin L	Papain
E-64	0.005	0.015	0.0025
Leupeptin	0.013	0.008	0.012
1	>63	nd	>63
2	4.81	nd	15.8
3	52	nd	>63
4	13.6	nd	57
5	>25	nd	>25
6	>25	nd	>25
7	0.47	0.042	0.275
8	1.46	0.030	0.731
9	42.29	2.70	0.228
10	0.47	0.035	nd

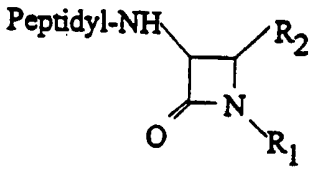
11	1.66	1.84	nd
12	7.4	1.58	nd
13	39.2	2.31	nd
14	24.5	1.29	nd
15	6.33	2.07	nd
16	5.68	0.035	nd
17	5.37	0.0315	nd
18	2.12	0.082	nd
19	7.22	0.416	
20	10.5	0.000108	nd
21	7.39	0.000126	nd
22	10.9	0.017	nd
23	7.01	0.163	nd
24	6.46	0.091	nd
25	11.4	0.78	nd
26	2.19	0.0556	nd
27	21.76	0.038	nd
28	0.076	0.228	nd
29A	0.59	0.16	nd
29B	>46	0.292	0.368
30B	>68	5.26	nd
31	8.43	0.067	nd
32	0.368	0.026	nd
33	14.31	35.9	7.03
34	0.33	0.0168	nd

nd = not determined

CLAIMS

We claim:

1. A 4-substituted-3-peptidyl-azetidin-2-one compound of formula I



I

wherein

R_1 is selected from the group consisting of;

C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; $-OR_3$ wherein R_3 is a C_1-C_6 alkyl which is unsubstituted or substituted by 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; and $-SO_3$-M^+ wherein M is hydrogen, a metal ion selected from the group consisting of sodium, potassium, magnesium, and calcium, or $N^+(R_4)_4$ wherein R_4 is C_1-C_6 alkyl;

R_2 is selected from the group consisting of;

C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; $-OCOR_5$ wherein R_5 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, heterocycle, or amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, or (v) phenyl which is

unsubstituted or substituted with 1-3 substituents selected from hydroxy, halogen, C_1-C_4 alkyl, C_1-C_2 alkoxy or cyano; $-XR_6$ wherein X is an O, S, SO,or SO_2 and R6 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, heterocycle, or amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) phenyl which is unsubstituted or substituted with 1-3 substituents selected from hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or substituted with at least one of carboxy and amino, C_1-C_2 alkoxy or cyano, or (vi) heterocycles;

Peptidyl is a 1-2 amino acid residue wherein the free NH_2 is unsubstituted or substituted with a protective group, or

a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 wherein the protective group is R_7 and is selected from the group consisting of:

$-COOR_8$ wherein R_8 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with phenyl, or (ii) phenyl; $-COR_9$ wherein R_9 is selected from the group consisting of (i) C_1-C_6 alkyl which is unsubstituted or substituted by 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, amino, 4-acetoxyphenyloxy, heterocycle, and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents selected from halogen, hydroxy, cyano, or amino, (ii) C_2-C_4 alkenyl is unsubstituted or substituted with heterocycle or phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents selected from halogen, hydroxy, cyano or amino, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) a phenyl group which is unsubstituted or substituted by 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or may be substituted with at least one of carboxy, or amino or both, C_1-C_2 alkoxy group or cyano, or

(vi) a heterocycle which may be mono or bicyclic; and -SO_2R_{10} wherein R_{10} is selected from the group consisting of (i) C_1-C_6 alkyl, (ii) C_2-C_4 alkenyl which is unsubstituted or substituted with heterocycle or phenyl, (iii) phenyl which is unsubstituted or substituted with 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl group, C_1-C_2 alkoxy group and cyano, and (iv) naphthyl which is unsubstituted or substituted by 1-3 substituents selected from hydroxy, halogen, cyano, carboxy, C_1-C_4 alkyl, or C_1-C_2 alkoxy.

3. A compound of claim 1 wherein the protective group for the free NH_2 is selected from the group consisting of aryloxy carbonyl, alkoxy carbonyl, substituted alkanoyl, arylalkanoyl, arylalkenoyl, heterocycloalkenoyl, heterocycloalkanoyl, alkylsulphonyl, arylsulphonyl, arylalkanylsulphonyl, arylalkensulphonyl, heterocycloalkanylsulphonyl, hetercycloalkensulphonyl, and heterocyclosulphonyl.

4. A compound of claim 1 wherein the heterocycle having 1-3 heteroatoms, wherein the heteroatoms are selected from the group consisting of nitrogen, sulphur, and oxygen, as substituent for R_5, R_6, R_9, and R_{10} are selected from the group consisting of thiophene, pyridine, 1,2,3-triazole, 1,2,4-triazole, quinoline, benzofuran, benzothiophene, morpholine, thiomorpholine, piperazine, and piperidine.

5. A compound of claim 1 wherein R_1 is selected from the group consisting of hydrogen, methoxy, 2-carboxy ethoxy, 2-aminoethoxy, 2-carboxy ethyl, 2-aminoethyl and sulphonic acid.

6. A compound of claim 1 wherein R_2 is selected from the group consisting of hydrogen, methyl, 2-amino ethyl, 2-carboxy ethyl, acetoxy, butyloxy, 3-methyl propyloxy, 1,1-dimethyl ethoxy, 2-carboxy ethyloxy, 2-aminoethyloxy, 2-fluoro ethoxy, 2-(1,2,3-triazol-4-yl)-ethoxy, cyclopentyloxy, cyclohexyloxy, cyclohexylthio, phenoxy, phenylthio, phenylsulphonyl, 4-(2-carboxy-2-amino

ethyl)-phenoxy, 4-carboxy phenoxy, 3-carboxy phenoxy, 2-pyridylthio, and 4-pyridylthio.

7. A compound of claim 1 wherein the Peptidyl is selected from the group consisting of phenylalanine, N-benzyloxy carbonyl phenylalanine, N-(3-phenyl propanoyl)-phenylalanine, N-acetyl phenylalanine, N-(2-(4-acetoxyphenoxy)- ethanoyl)-phenylalanine, N-(morpholin-4-yl-carbonyl)-phenylalanine, N-(3- (morpholin-4-yl)-propanoyl)-phenylalanine, N-(3-(pyridin-3-yl)-propanoyl)-phenylalanine, N-(benzofuran-2-yl-carbonyl)-phenylalanine, N-(3-(thiophen-2-yl)-prop-2-enoyl)-phenylalanine, N-(4-(1,1-dimethyl ethyl phenyl)-sulphonyl)-phenylalanine, N-(naphthalen-2-yl-sulphonyl)-phenylalanine, N-(3-phenyl-prop-2-en-sulphonyl)-phenylalanine, N-benzyloxy carbonyl leucine, N-benzyloxy carbonyl isoleucine, N-3-phenyl propanoyl leucine, N-3-phenyl propanoyl isoleucine, N-benzyloxy carbonyl proline, and N-benzyloxy carbonyl phenylalanine-glycine.

8. A compound of claim 1 having (3R,4S), (3R,4R), (3S,4R) or (3S,4S) configuration at the two asymmetric carbons 3 and 4 on the azetidin-2-one ring system, or a racemic mixture thereof.

9. A compound of claim 1 wherein the Peptidyl group is a 1-2 amino acid residue having D, L isomers, or a racemic mixture thereof.

10. A compound of claim 1 wherein the Peptidyl group is a 1 amino acid residue.

11. A compound of claim 1 wherein the Peptidyl group is a 2 amino acid residue.

12. A compound of claim 1 wherein the pharmaceutically acceptable salts are selected from the group consisting of sodium, potassium, magnesium, calcium, hydrogen chloride, tartaric acid, succinic acid, and fumaric acid p-toluenesulfonic acid or the like.

13. A compound of claim 1 selected from the group consisting of:

(3S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-1-methoxy-azetidin-2-one;

(3S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-azetidin-2-one;

(3R)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-1-methoxy-azetidin-2-one;

(3R)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-methyl-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl-glycyl)-amino-4-methyl-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-leucyl)-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-(N-acetyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(trans-3-phenylpropenoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(morpholin-4-yl-carbonyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-morpholin-4-yl-propionoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-pyrid-3-yl-propionoyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-[N-{2-(4-acetoxyphenoxy)-ethnoyl}-L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(benzofuran-2-yl-carbonyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-[N-{3-(thiophen-2-yl)-trans-prop-2-enoyl}-L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-[N-{4-(1,1-dimethyl ethyl phenyl)-sulfonyl}-L-phenylalanyl]-amino-4-acetoxy-azetidin-2-one;

(3S,4S)-3-{N-(naphthalen-2-yl-sulfonyl)-L-phenylalanyl}-amino-4-acetoxy-azetidin-2-one;

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one;

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylsulfonyl-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenoxy-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-butyloxy-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(2-methyl propyloxy)-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(1,1-dimethylethoxy)-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-phenoxy-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-(4-diphenylmethoxy carbonylphenoxy)-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-(4-carboxyphenoxy)-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-(3-carboxyphenoxy)-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-{4-(L-2-benzyloxy-carbonylamino-2-diphenylmethoxycarbonyl ethyl)-phenoxy}-azetidin-2-one;

(3S,4S)-3-{N-(3-phenylpropionoyl)-L-phenylalanyl}-amino-4-{4-(L-2-amino-2-carboxy ethyl)-phenoxy}-azetidin-2-one;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(4-diphenylmethoxycarbonyl phenoxy)-azetidin-2-one;

(3S,4S)-3-(L-phenylalanyl)-amino-4-(4-carboxyphenoxy)-azetidin-2-one;

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-phenylthio-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-acetoxy-azetidin-2-one-1-sulfonic acid;

(3S,4S)-3-(N-benzyloxycarbonyl-L-alanyl)-amino-4-acetoxy-azetidin-2-one; and

(3R,4S)-3-(N-benzyloxycarbonyl-L-phenylalanyl)-amino-4-(period-4-yl-thio)-azetidin-2-one.

14. A pharmaceutical composition suitable for the treatment of muscular dystrophy, bone resorption, myocardial infarction, and cancer metastasis, comprising the compound of claim 1 in an amount effective to inhibit cysteine proteinase and a pharmaceutically acceptable excipient.

15. A method of treatment of muscular dystrophy comprising administering an effective amount of the compound of claim 1 to a mammal in need of such treatment.

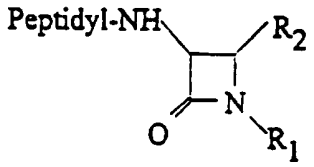
16. A method of treatment of disturbances of bone resorption comprising administering an effective amount of the compound of claim 1 to a mammal in need of such treatment.

17. A method of treatment of myocardial infarction comprising administering an effective amount of the compound of claim 1 to a mammal in need of such treatment.

18. A method of treatment of cancer metastasis wherein the cancers are selected from the group consisting of breast, lung, liver, colon, brain, and prostate, comprising administering an effective amount of the compound of claim 1 to a mammal in need of such treatment.

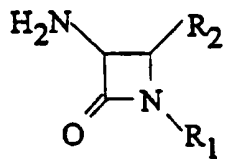
19. A method of inhibiting cysteine proteinases in a mammal comprising administering an effective amount of the compound of claim 1 to a mammal in need of such treatment.

20. A process for preparing 4-substituted-3-peptidyl-azetidin-2-one derivatives of formula I



I

comprising reacting a compound of formula II



II

with a 1-2 amino acid peptidyl-OH in which the free NH_2 of the peptidyl is unsubstituted or substituted with a protective group R_7

wherein

R_1 is selected from the group consisting of hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; $-OR_3$ wherein R_3 is a C_1-C_6 alkyl which is unsubstituted or substituted by 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; and $-SO_3$-M^+ wherein M is hydrogen, a metal ion selected from the group consisting of sodium, potassium, magnesium, and calcium, or $N^+(R_4)_4$ wherein R_4 is C_1-C_6 alkyl;

R_2 is selected from the group consisting of hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; -$OCOR_5$ wherein R_5 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, heterocycle, or amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, or (v) phenyl which is unsubstituted or substituted with 1-3 substituents selected from hydroxy, halogen, C_1-C_4 alkyl, C_1-C_2 alkoxy or cyano; $-XR_6$ wherein X

is an O, S, SO,or SO_2 and R6 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, heterocycle, or amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) phenyl which is unsubstituted or substituted with 1-3 substituents selected from hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or substituted with at least one of carboxy and amino, C_1-C_2 alkoxy or cyano, or (vi) heterocycles, and

R_7 is selected from the group consisting of -$COOR_8$ wherein R_8 is (i) C_1-C_6 alkyl which is unsubstituted or with phenyl, or (ii) phenyl; -COR_9 wherein R_9 is selected from the group consisting of (i) C_1-C_6 alkyl which is unsubstituted or substituted by 1-2 substituents selected from the group consisting of hydroxy, halogen, cyano, amino, 4-acetoxyphenyloxy, heterocycle, and phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents selected from halogen, hydroxy, cyano, or amino, (ii) C_2-C_4 alkenyl is unsubstituted or substituted with heterocycle or phenyl, wherein the phenyl is unsubstituted or substituted by 1-2 substituents selected from halogen, hydroxy, cyano or amino, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) a phenyl group which is unsubstituted or substituted by 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or may be substituted with at least one of carboxy, or amino or both, C_1-C_2 alkoxy group or cyano, or (vi) a heterocycle which may be mono or bicyclic; and -SO_2R_{10} wherein R_{10} is selected from the group consisting of (i) C_1-C_6 alkyl, (ii) C_2-C_4 alkenyl which is unsubstituted or substituted with heterocycle or phenyl, (iii) phenyl which is unsubstituted or substituted with 1-3 substituents selected from the group consisting of hydroxy, halogen, carboxy, C_1-C_4 alkyl group, C_1-C_2 alkoxy group and cyano, and (iv) naphthyl which is unsubstituted or substituted by

1-3 substituents selected from hydroxy, halogen, cyano, carboxy, C_1-C_4 alkyl, or C_1-C_2 alkoxy.

21. The process of claim 20 wherein R_7 is $-COOR_8$ comprising:
reacting the free NH_2 peptidyl group with R_8OCl.

22. The process of claim 20 wherein R_7 is $-COR_9$ comprising
reacting the free NH_2 peptidyl group with either: a) R_9-COOH in the presence of DCC, b) R_9COCl in the presence of base, c) $(R_9CO)_2O$ (anhydride) in the presence of base, or d) an activated ester of R_9COOH.

23. The process of claim 20 wherein R_9 is $-SO_2R_{10}$ comprising
reacting the free NH_2 peptidyl group with $R_{10}SO_2Cl$ in the presence of base.

24. The process of claim 20 wherein R_2 is XR_6, wherein X is O or S, comprising:
a) providing a compound of formula I wherein R_2 is $OCOCH_3$;
b) reacting said compound with R_6XH in the presence of a lewis acid.

25. The process of claim 24 wherein the lewis acid is selected from the group consisting of zinc acetate, zinc iodide, zinc chloride, titanium tetrachloride, palladium acetate, boron trifluoride, and aluminum trichloride.

26. The process of claim 24 wherein a) a carboxy group as substituent in R_6 is protected with an R_{11} selected from the group consisting of diphenyl methyl and 1,1-dimethyl ethyl, or b) an amino group as substituent in R_6 is protected with an R_{12} selected from the group consisting of benzyloxy carbonyl and 1,1-dimethyl ethoxy carbonyl, or c) both protected groups as substituents in R_6 together were deprotected by hydrogenation or hydrolysis with acids.

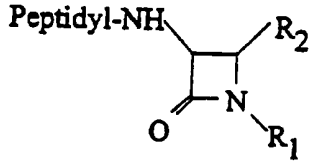
27. The method of claim 20 wherein R_2 is SOR_6 or SO_2R_6 comprising converting a compound of formula I wherein R_2 is SR_6

to a compound of formula I wherein R_2 is SOR_6 or SO_2R_6 comprising oxidating with an oxidizing agent selected from the group consisting of m-chloroperbenzoic acid, hydrogen peroxide, peracetic acid, potassium permanganate, and manganese dioxide.

28. The method of claim 20 wherein R_1 is SO_3H comprising converting a compound of formula I wherein R_1 is hydrogen to N-sulphonic acid by sulphonation with pyridine-SO_3 or dimethylformamide-SO_3 complex.

29. The method of claim 20 further comprising converting a compound of formula I wherein R_2 = $OCOCH_3$ to compounds wherein R_2 is other substituents by reacting said compound with substituted hydroxy or thiol compounds at a temperature between -40 and 150°.

30. A 4-substituted-3-peptidyl-azetidin-2-one compound of formula I



I

wherein

R_1 is selected from the group consisting of hydrogen; C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; $-OR_3$ wherein R_3 is a C_1-C_6 alkyl which is unsubstituted or substituted by 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; and $-SO_3$-M^+ wherein M is hydrogen, a metal ion selected from the group consisting of sodium, potassium, magnesium, and calcium, or $N^+(R_4)_4$ wherein R_4 is C_1-C_6 alkyl;

R_2 is selected from the group consisting of hydrogen;

C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, carboxy or amino; -$OCOR_5$ wherein R_5 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, heterocycle, or amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, or (v) phenyl which is unsubstituted or substituted with 1-3 substituents selected from hydroxy, halogen, C_1-C_4 alkyl, C_1-C_2 alkoxy or cyano; -XR_6 wherein X is an O, S, SO,or SO_2 and R6 is (i) C_1-C_6 alkyl which is unsubstituted or substituted with 1-2 substituents selected from hydroxy, halogen, cyano, heterocycle, or amino, (ii) C_2-C_4 alkenyl, (iii) C_2-C_4 alkynyl, (iv) C_3-C_6 cycloalkyl, (v) phenyl which is unsubstituted or substituted with 1-3 substituents selected from hydroxy, halogen, carboxy, C_1-C_4 alkyl which is unsubstituted or substituted with at least one of carboxy and amino, C_1-C_2 alkoxy or cyano, or (vi) heterocycles;

Peptidyl is a 1-2 amino acid residue wherein the free NH_2 is unsubstituted or substituted with a protective group, or

a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

Inter. nal Application No
PCT/IB 96/00268

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K5/06 C07K5/08 A61K38/55 A61K31/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C07D A61K C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 393 457 (SUNTORY LIMITED) 24 October 1990 ---	
A	US,A,5 223 486 (S.G. GORDON ET AL.) 29 June 1993 ---	
A	WO,A,93 18063 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 16 September 1993 ---	
A	GB,A,2 227 411 (SANDOZ LTD.) 1 August 1990 -----	

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
4 June 1996	13.06.96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Chouly, J

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INTERNATIONAL SEARCH REPORT

...formation on patent family members

Intern al Application No
PCT/IB 96/00268

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